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SCREENING AND OPTIMISING METAL SALT CONCENTRATION FOR HARVESTING *NANNOCHLOROPSIS* Sp. BY FLOCCULATION

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ABSTRACT

Biodiesel is a renewable fuel that is non-toxic, biodegradable, and constantly increasing in demand as the reservoir of fossil fuel is depleting. The microalgae biomass with high oil content is significant as a sustainable resource for biodiesel production. Production of biodiesel using microalgae biomass appears to be a feasible alternative due to no confliction with food supply compared with the first generation biofuels. This report deals with the screening and optimisation of metal salts for harvesting marine microalgae by flocculation. The metal salts studies are ferric chloride, aluminium sulphate and ferric sulphate. Wild Nannochloropsis strains of microalgae were cultivated aseptically in seawater for seven days, after that the microalgae was harvested by using flocculation step with different concentration of metal salt. In order to monitor the efficiency of the metal salt, the turbidity region of microalgae in glass cylinder before and after flocculation was observed. Besides that cell, dry weight was also compared for three-flocculation agent used. The most efficient metal salt was then further optimized for its best-performed concentration and pH. Chloride salts ($FeCl_3$) was found to be more efficient in comparison with sulfate salts ($Al_2(SO_4)_3$ and $Fe_2(SO_4)_3$) in harvesting microalgae. Ferric Chloride was further optimized, where the optimum pH and concentration of $FeCl_3$ are 8.5 M and 1.0 M, with flocculation efficiency of 90 % and cell dry weight of 3.8 g. In conclusion, 1.0 M ferric chloride salt at pH 8.5 is optimum in harvesting Nannochloropsis sp. microalgae by flocculation.

KEYWORDS**:** *Microalgae; flocculation; pH; nannochloropsis sp.; biodiesel*

1.0 INTRODUCTION

In the wake of diminution in fossil fuel reservoir, heaps of research exploring alternative fuel has been done. Biodiesel that emits much lower toxic air may be an option in resolving this problem. Consequently, it has become constantly increase in demand. Among the alternatives studied in producing biodiesel, microalgae have been proposed to be the potential solution to defeat this crisis (Chisti, 2007; Khan, Rashmi, Hussain, Prasad & Banerjee, 2009; Mata, Martin & Caetano, 2010; Singh & Gu, 2010). As

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compared to crops, microalgae have a higher productivity and higher oil content, which is up to 80% on dry weight (Raja, Hemaiswarya, Ashok Kumar, Sridhar & Rengasamy, 2008; Converti, Alessandro, Erika, Patrizia & Marco, 2009). Hence, it is obvious that microalgae can be an attractive option on large-scale fuel oils production.

In order to become much more appealing choices, biodiesel production must offer a lower or at least the same price as existing fossil fuels. Nonetheless, accomplishing the goal required consideration on the microalgae biodiesel value chain such as microalgae strain, their cultivation unit and methods, site selection, harvesting approach and final biomass concentration, components extraction and biodiesel production (Khan et al., 2009; Mata et al., 2010). Any improvement of these value chain components will certainly lower the price of microalgae biodiesel.

Harvesting of microalgae biomass involves one or more solid–liquid separation steps. Existing technologies such as centrifugation, filtration, dissolved air floatation, and sedimentation are well suited to harvest small particles from bulk liquid (Molina Grima, Blarbi, Acién Fernández, Robles Medina & Chisti, 2003). Yet, these processes are not practical for harvesting lipid-rich microalgae as they are either too energy or chemical-intensive or require too much time (Oh et al., 2001; Papazi, Makridis & Divanach, 2010).There is no single best method for harvesting microalgae. Factors such as algae species, growth medium, algae production rate, product characteristics and production cost-benefit would determine the harvesting technology to be used (Subcontract Report No.XK-3-03031-01, 1984). Flocculation is normally used before these processes to facilitate efficient harvesting. Numerous flocculation methods could be applied in microalgae biomass recovery. This step intended to aggregate the microalgae cells in order to form particles with effective size that simplifying the filtration or centrifugation processes. Ideally, a cheap and non-toxic flocculants that is able to perform effectively in a dilute broth and do not impose any adverse effect on the subsequent downstream process shall be chosen. Harvesting of algae using flocculation is very convenient as it can treat a large quantity of microalgae biomass in a short time.

In view of the above, various studies have been done in searching an effective flocculant. Multivalent metal salts and cationic polymers are reported to be effective flocculants or coagulants (Granados, Acién, Gómez, Fernández-Sevilla & Molina Grima, 2012; Vandamme, Foubert & Muylaert, 2013; Roselet, Vandamme, Roselet, Muylaert & Abreu, 2015). Metal salts such as aluminium sulphate, ferric chloride and ferric sulphate are generally preferred in flocculation processes as they lead to enhancedharvesting efficiency in most medium (Molina Grima et al., 2003; Papazi et al., 2010). Cationic polymers, however, are less effective for marine algae that are cultured in high salinity medium as compared to metal salts (Bilanovic, Shelef & Sukenik, 1988; Molina Grima et al., 2003; Vandamme, Foubert, Meesschaert & Muylaert, 2010). This is attributed to the high ionic strength of the medium that competing with cationic polymers thus renders the polymers useless.

Generally, the choice of metal salts and their optimum concentration are dependent on the type of cells, their culture medium, the purpose of the process and the targeted product

(Molina Grima et al., 2003; Papazi et al., 2010). Since our target product is biofuel, *Nannochloropsis* sp. that is one of the highest biofuel producers (Chisti, 2007) was chosen as a model of study. Bio flocculation (Surendhiran & Vijay, 2014) and magnetic nanoparticles (Hu, Wang, Wang, Liu & Guo, 2013) have been reported as two of the effective techniques in harvesting *Nannochloropsis* sp. However, these methods are either expensive or require special handling technique. Şirin and Sillanpää (2015) reported that they used natural sedimentation and pH induced flocculation to harvest *Nannochloropsisoculata* in municipal wastewaters. Though the method is cheaper and easier, the flocculation efficiency is only about 80%, which is not as good as using metal salts. Hence, the objectives of this work are to screen an efficient metal salt for harvesting marine microalgae *Nannochloropsis* sp. by flocculation and to optimise the concentration of metal salt and pH in the flocculation step.

2.0 MATERIALS AND METHODS

All chemicals were purchased from Sigma-Aldrich and R&M Chemicals unless otherwise stated.

2.1 Microalgae Culture

The marine microalgae *Nannochloropsis sp.* was collected from University Malaysia Terengganu. The microalgae were cultured in 14 parallel one-liter conical flasks aerated with filtered air and cultivated at 22±2ºC, 2000 lux illumination intensity, and 12:12 hours photoperiods for seven days. Standard F/2 medium was used to culture the microalgae and the inoculation volume is 30% (v/v). All media and apparatus were sterilized prior to experimentation. Heat labile components were filter-sterilized and all transfer processes were run aseptically in a biosafety cabinet.

2.2 Flocculation Experiment

After inoculation, microalgae were left to grow for seven days. Microalgae were then harvested by flocculation step at day 7. Ferric chloride [$FeCl_3$], aluminium sulphate [$Al_2(SO_4)_3$] and ferric sulphate [$Fe_2(SO_4)_3$] were used as the flocculation agents in this study. Eight hundred microliters of $FeCl_3$at various concentrations (0.25 M,0.5 M, 0.75 M, 1.0 M and 1.25 M) was added to 150 ml of microalgae cultures in each 250 mL size glass measuring cylinder. The mixture was magnetically stirred for 20 seconds after pH adjustment to approximately 8 M with 1.0 M NaOH. The mixtures were then being left standing for two hours to enable flocculating and settling process. After two hours, the turbidity region were observed, measured and recorded to calculate the flocculation efficiency of each flocculation agent. Similar procedure was repeated for $Al_2(SO_4)_3$ and $Fe_2(SO_4)_3$.

2.3 Measurement of Flocculation Efficiency

The flocculation efficiency was evaluated by comparing the clear region of microalgae culture in the glass measuring cylinder after and before adding flocculants. Flocculation efficiency (FE) was calculated using Equation (1) below.

$$Flocculation\ Efficiency = \left(\frac{Ri - Rf}{Ri} \right) \times 100 \quad (1)$$

where *Ri* is the initial turbidity region before treatment and *Rf* is the turbidity region after flocculation (Ding & Salihon, 2011).

2.4 Measurement of Cell Dry Weight

The solution above the precipitated microalgae was decanted carefully. The remainder microalgae flocculant was then centrifuged (Eppendorf 5810R, Germany) at 5000 rpm for five minutes to remove the residue liquid. The cell pellet was dried in the oven (Memmert, Germany) for 24 hours at 60°C to get the cell dry weight of microalgae harvested. The weight was measured several times until constant value was obtained.

2.5 Effect of Different pH and Concentration

The effect of different pH and the optimum concentration of $FeCl_3$ (most efficient flocculation agent from the result of flocculation experiment; see section 3.1) were investigated further. The effect of different pH was conducted by adding 1.0 M NaOH drop by drop until it reach the pH that was set for this experiment (pH 5.5, 6.5, 7.5, 8.5 and 9.5) using pH meter. Similar pH range was tested using a different concentration of $FeCl_3$ (0.9, 1.0 and 1.1 M). The same procedure was repeated as per Section 2.3 and 2.4 above to observe the flocculation efficiency and cell dry weight of the microalgae.

3.0 RESULTS AND DISCUSSION

3.1 Screening Of Metal Salts For Efficient Harvesting Of Microalgae

Figure 1 shows the results of flocculation efficiency in harvesting microalgae using three different metal salts at various concentrations. The flocculation efficiency increased when the concentration of $FeCl_3$ increased. It attained a maximum of 99.3% flocculation efficiency at the concentration of 1.0 M. Further increased in $FeCl_3$ concentration caused a decrease of flocculation efficiency. $Fe_2(SO_4)_3$ shows a rather similar trend. The maximum flocculation efficiency when using $Fe_2(SO_4)_3$ as a flocculant also occurred at 1.0 M concentration. Aluminium Sulphate, on the other hand, shows a slightly different trend where the highest flocculation efficiency of 97.9 % was achieved at 0.5 M. There is a drop in flocculation efficiency if concentration of $Al_2(SO_4)_3$ was increased above 0.5 M. According to Vandamme et al. (2010), a drop in the flocculation efficiency after exceeding certain flocculant concentration may be due to the effect of steric hindrance

and/or electrostatic repulsion. When too much cation adheres on the surface of the cells, it shields the cells from meeting each other to form aggregate in addition to repulsion.

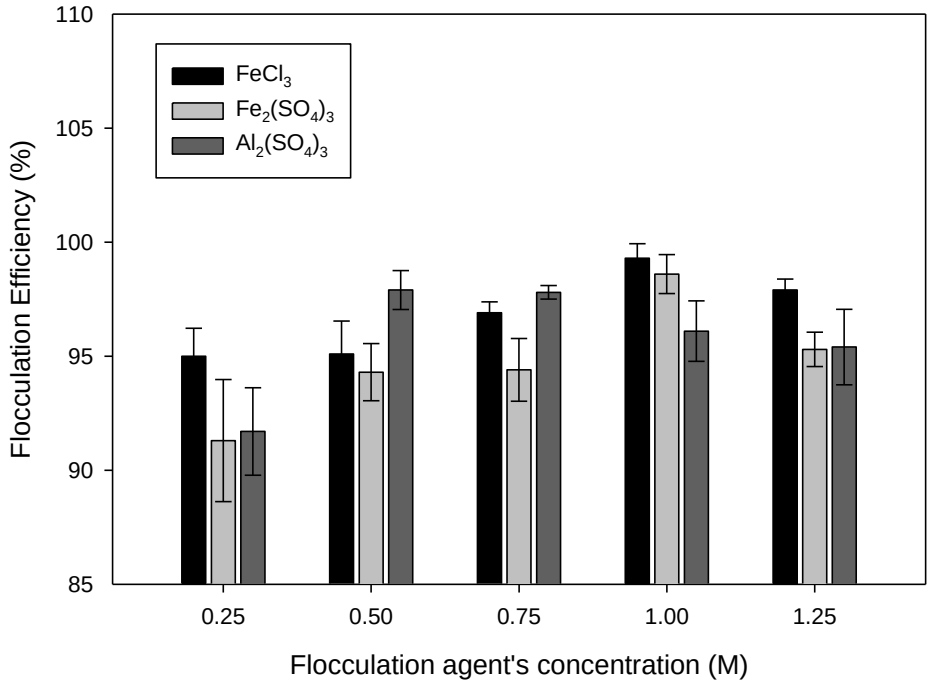

Figure 1. Comparison of flocculation efficiency (%) between different metal salts at various concentrations

Figure 2 depicts the effect of varying metal salts concentration on the cell dry weight of microalgae obtained. Among the metal salts tested, $FeCl_3$ at 1.0 M concentration attained the highest cell dry weight (0.0791$\pm$ 0.00374 g). The maximum cell dry weight achieved by $Fe_2(SO_4)_3$ was 0.0681$\pm$0.0010 g at 1.0 M concentration, whereas the highest cell dry weight obtained with $Al_2(SO_4)_3$ was occurred at 0.5 M, which was 0.0587$\pm$0.0041g. From the results, it is obvious that aluminium salts precipitate lesser microalgae cells than ferric salts. The maximum cell dry weight obtained was consistent with the highest flocculation efficiency. This finding was contradicted with that had been quoted by Oh et al. (2001) and Molina Grima et al. (2003). Oh et al. (2001) mentioned that aluminium sulphate is the most effective flocculants, following by certain cationic polyelectrolytes. However, these results were in line with the finding of Papazi et al. (2010), where they found that chloride salts were more efficient as compared to sulphate salts in flocculating the algae cells. Cell aggregate formation was immediately observed after addition of appropriate amount of chloride salts, which was similar to the observation of present work. According to Papazi et al. (2010), chloride salts are more soluble in wider concentration range than sulphate salts; thus, aid in flocculating and precipitating the algae flocculant.

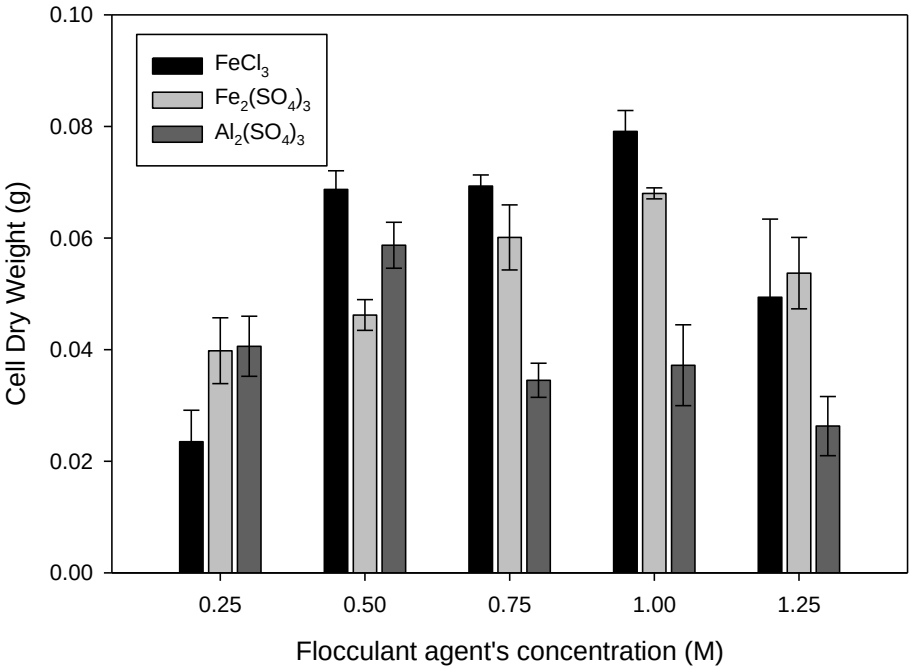

Figure 2. Comparison of cell dry weight (g) between different metal salt at various concentrations

Ferric salts were observed to cause a change in the color of the cells from green to brown in the present work. Nonetheless, no color changes were noticed in aluminium salts (data not shown). Papazi et al. (2010) reported that ferric salts affect the pigmentation of microalgae at the concentration above 1 g/L. In current work, the minimum concentration tested was 0.25 M, which is corresponding to 40.55 g/L of $FeCl_3$ and 99.97 g/L of $Fe_2(SO_4)_3$. Thus, the concentration employed is well above the minimum threshold. As a result, the changes in culture color were observed. Considering the purpose of *Nannochloropsis*sp. is not for pigment production, this effect is not significant and can be ignored.

Microalgae cells repel each other and exist as a single cell in the culture mediumas a result of the negative charges on the cells' surface (Molina Grima et al., 2003; Knuckey, Brown Robert & Frampton, 2006). When metal salts flocculation agent was dissolved in the medium,they dissociated to their respective ions. The cation dissociated from the flocculant added will adsorb on the surface of the cells. This has brought to surface charge neutralization, where the net electrical charge of the microalgae particle has been cancelled with an equal amount of the opposite charge. As a consequence of the interactions, cell aggregates formed (Molina Grima et al., 2003; Knuckey et al., 2006; Papazi et al., 2010). The higher the activity or electronegativity will result in higher efficiency of cell aggregation. Ferrousis obviously had a higher electronegativity then aluminium. Hence, the resultant higher flocculation efficiency. Since ferric chloride gave

the highest flocculation efficiency and the greatest amount of microalgae cell dry weight, it has been chosen to further optimized as in the Section 3.2.

3.2 Optimizing pH and concentration of $FeCl_3$ for efficient harvesting of microalgae

In this section, the range of concentration selected was based on the optimum region obtained in the previous section, where $FeCl_3$ was determined to be the best flocculant in harvesting microalgae. The pH of the medium was found to give significant effect on the flocculation efficiency of microalgae (Oh et al., 2001; Molina Grima et al., 2003; Knuckey et al., 2006; Tuan Harith, Mohd Yusoff, Mohamed, Mohamed Din & Ariff, 2009; Papazi et al., 2010; Vandamme et al., 2010; Sirin, Trobajo, Ibanez & Salvadó, 2011; Wu et al., 2012). Most reports revealed that if the culture medium is slightly alkaline at about pH8 to pH11, efficient flocculation would occur irrespective of the flocculant type.

It can be seen from Figure 3 that flocculation efficiency achieved an optimum around pH 7.5 for nearly all $FeCl_3$ concentration tested**.** The structure of the flocs formed at this pH value was slightly different than those produced at lower pH levels. The flocs looked more robust and larger. They also settled more rapidly.The optimum flocculation efficiency at 1.0 M $FeCl_3$ is somewhat different. It occurred at pH range of 7.5 - 8.5 (~90%), which was slightly alkaline than those for 0.9 M and 1.1 M. At pH 9.5, an obvious reduction in flocculation efficiency was observed for all concentrations of $FeCl_3$, but a declining trend for 0.9 M and 1.1 M $FeCl_3$ started earlier at pH8.5. Knuckey et al., (2006) reported that when pH adjustment was done exceeding the optimal value, algal flocculation will not be improved. On top of this, calcium carbonate and magnesium hydroxide formation may occur at high pH; thus, increased the bulk precipitate (Knuckey et al., 2006; Wu et al., 2012; Vandamme et al., 2013). As a result of this, loose flocs that did not pack tightly when settled were formed (Knuckey et al., 2006; Sirin et al., 2011) and occupied a greater volume of precipitating region. This in turn affected the measurement of the height of turbidity region; hence, the calculation of flocculation efficiency.

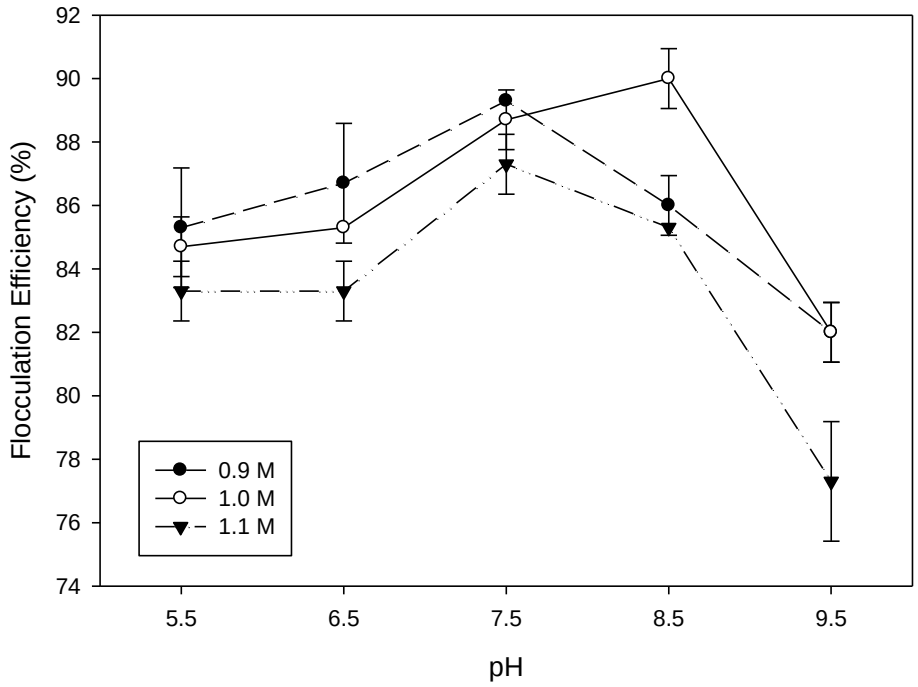

Figure 3. Flocculation efficiency (%) of $FeCl_3$ at different pH and concentrations

Figure 4 depicts the relation between cell dry weights with pH at different concentration of $FeCl_3$. At 0.9 M and 1.1 M of $FeCl_3$, pH7.5 leads to the highest cell dry weight of 3.53 g and 3.47 g respectively. In contract, a comparable cell dry weight (3.79 g) was obtained at pH 8.5 for the concentration of 1.0 M. This result is consistent to that of flocculation efficiency. When flocculation efficiency increased, more algae cells would be precipitated. As a result, more biomass could be harvested, which would increase the cell dry weight obtained. As mentioned above, loose flocs were formed at pH8.5 and above, which composed of algae cells, calcium carbonate and magnesium hydroxide. Along the process of washing the precipitate, it was possible that calcium carbonate and magnesium hydroxide were being washed out, leaving only algae cells (Knuckey et al., 2006). As a result, the cell dry weight obtained was reduced. Most waters contain sufficiently high background concentration of magnesium (Vandamme et al., 2013). When pH is high, formation of magnesium hydroxide precipitate occurred. Similarly, ferric chloride that was added also readily form ferric hydroxide at pH greater than 4 (Wyatt, Gloe, Brady, Hewson, Grillet, Hankins & Pohl, 2011). These precipitates carry a positive surface charge, so it will induce flocculation through charge neutralization. However, when pH is too high (e.g. pH10), all surfaces are negatively charged (cell surface, magnesium hydroxide, ferric hydroxide); thus, electrostatic repulsion occurs and less cells can be flocculated.

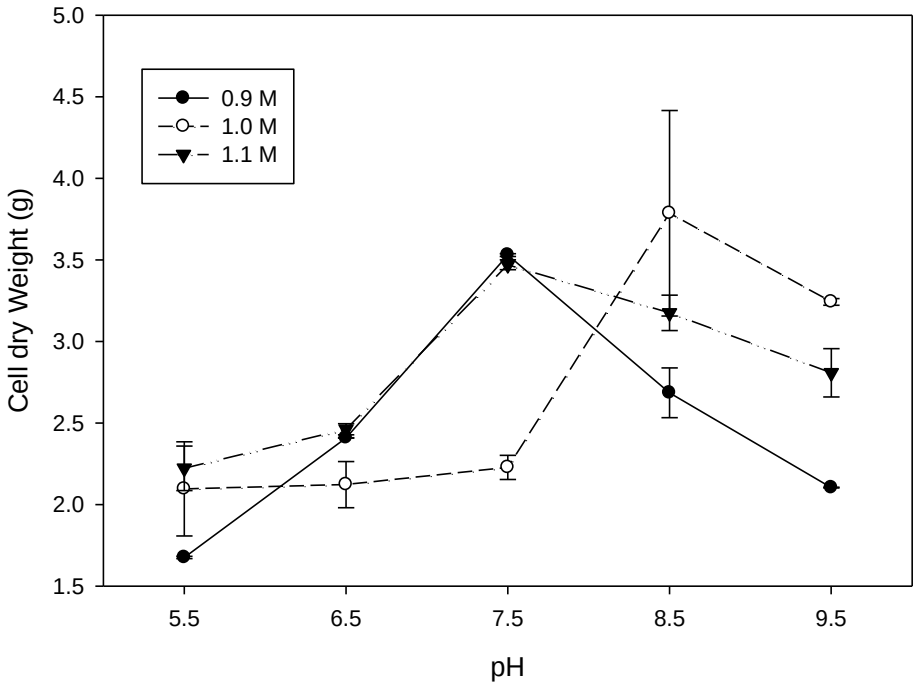

Figure 4. Cell dry weight (g) obtained at different pH and concentrations of $FeCl_3$

Sirin et al. (2011) investigated harvesting of *Phaeodactylum tricornutum* with polyaluminum chloride, aluminium sulphate and chitosan. They found that each flocculant performance well at different pH. The optimal pH for aluminium sulphate (30 mg/L) was between pH5 and 9, polyaluminum chloride (30 mg/L) at near pH5 and 7, while optimal pH for chitosan (20 mg/L) was at 9.9. In harvesting dense culture of *Chlorella minutissima*, Papazi et al., (2010) tested twelve type of salts without control of initial medium pH. Sulfate and chloride salts for aluminum, ferric and zinc were found to be most optimum and gave highest flocculation efficiency at the concentration of 0.75 and 0.5 g/L, respectively. Addition of non-ionic polymer Magnafloc LT-25 at 0.5 mg/L and pH culture of between 10 - 10.6, successfully flocculated and harvested cells of *Chaetoceros calcitrans, Chaetocerosmuelleri, Thalassiosira pseudonana, Attheya septentrionalis, Nitzschia closterium, Skeletonema* sp., *Tetraselmis suecica* and *Rhodomonas salina*, with efficiencies greater than 80% (Knuckey *et al.*, 2006). In the study of Tuan Harith et al., (2009), 0.1 mg/L Magnafloc LT25 and LT27 gave the optimal flocculation efficiency of *Chaetoceros calcitrans*at pH8 and pH10.2 respectively. Without adding any flocculant, Wu et al., (2012) examined flocculation of *Nannochloropsis oculata* and *Phaeodactylum tricormutum* using pH adjustment. They reported that greater than 90% flocculation efficiency was achieved at pH9 and pH9.3 for *Nannochloropsis oculata* and *Phaeodactylum tricormutum*, respectively. Since the culture medium used in their study (ASW, modified artificial seawater) was different from the present study (F/2 medium), it might be the reason for the diverse in observation,

where no flocculation happen when only NaOH was added (data not shown). It seems that the optimal conditions for maximizing algal biomass recovery are dependent on many factors such as cell surface properties of the strains, culture conditions and composition of the medium (Vandamme et al., 2013). Therefore, further investigation considering all aspects must be done before an economic and efficient method could be determined.

4.0 CONCLUSION

Based on the screening experiment conducted, chloride salts ($FeCl_3$) was more efficient in comparison with sulphate salts ($Al_2(SO_4)_3$ and $Fe_2(SO_4)_3$) for harvesting the marine microalgae *Nannochloropsissp*. $FeCl_3$ gives the highest flocculation efficiency and cell dry weight at 1.0 M concentration of $FeCl_3$. In optimizing concentration of $FeCl_3$, pH8.5 and 1.0 M $FeCl_3$ were determined to be the optimum conditions. These were selected as it gave the highest flocculation efficiency of 90% and cell dry weight of 3.80 g. Further investigation (such as culture conditions, medium composition and sedimentation rate) needs to be done before economic production of biofuel from marine microalgae oil becomes a reality. For future work, it is recommended that the effect of metal salt ($FeCl_3$) on the cost and downstream processing of oil extraction shall be investigated in order to determine most time and cost saving of harvesting method in large-scale microalgae biofuel production.

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